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# Combined Bicarbonate Conductance-Impairing Variants in *CFTR* and *SPINK1* Are Associated with Chronic Pancreatitis in Patients without Cystic Fibrosis

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## Abstract

**Background & Aims—**Idiopathic chronic pancreatitis (ICP) is a complex inflammatory disorder associated with multiple genetic and environmental factors. In individuals without cystic fibrosis (CF), variants of *CFTR* that inhibit bicarbonate conductance but maintain chloride conductance might selectively impair secretion of pancreatic juice, leading to trypsin activation and pancreatitis. We investigated whether sequence variants in the gene encoding the pancreatic secretory trypsin inhibitor, *SPINK1*, further increase the risk of pancreatitis in these patients.

**Methods—**We screened patients with ICP (sporadic or familial) and controls for variants in *SPINK1* associated with chronic pancreatitis (CP) risk (in exon 3) and in all 27 exons of *CFTR*. The final study group included 53 patients with sporadic ICP, 27 probands with familial ICP, and 150 unrelated controls, plus 503 controls for limited genotyping. *CFTR* wild-type (wt) and p.R75Q were cloned and expressed in HEK293 cells and relative conductances of $HCO3^-$ and $Cl^-$ were measured.

**Results—***SPINK1* variants were identified in 36% of subjects and 3% controls (odds ratio [OR]=16.5). One variant of *CFTR* that has not been associated with CF, p.R75Q, was found in 16% of subjects and 5.4% controls (OR=3.4). Co-inheritance of *CFTR* p.R75Q and *SPINK1* variants occurred in 8.75% of patients and 0.15% controls (OR=62.5). Patch-clamp recordings of cells that expressed *CFTR* p.R75Q demonstrated normal chloride currents but significantly reduced bicarbonate currents (*P*=0.0001).

**Conclusions—**The *CFTR* variant p.R75Q causes a selective defect in bicarbonate conductance and increases risk for pancreatitis. Co-inheritance of CF-associated, and some not associated, *CFTR* variants with *SPINK1* variants significantly increase risk of ICP.

### Keywords

NAPS2; pancreas; polygenic; risk factor; patch-clamp; epistasis

## Introduction

Chronic pancreatitis (CP) is a complex and highly variable inflammatory syndrome defined by episodes of acute pancreatitis, chronic pancreatic inflammation, progressive fibrosis, loss of pancreatic exocrine and endocrine function and chronic pain [1–3]. The most common etiology of pancreatitis in children is cystic fibrosis (CF) [4], an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*).

CFTR is an epithelial cell anion channel that is expressed in the pancreas, airways, gastrointestinal track, biliary tree, male reproductive organs and sweat gland and is critical for normal fluid secretion. There are over 1600 known mutations of *CFTR* that cause CF, the pancreatic severity of which is determined primarily from the category of mutation that has been identified in patients [5]. Patients who carry two *CFTR* mutations categorized by

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phenotype as CF-severe and a by molecular classification as Class I or II, with near total loss of either mRNA or protein expression usually develop classic CF [6]. These patients present with advanced chronic pancreatitis, malabsorption and failure to thrive in the first few years of life and have pancreatic insufficiency with an inability to maintain nutritional needs without pancreatic enzyme supplements at, or soon after diagnosis. CF Patients carrying at least one CTR mutation categorized by phenotype as CF-mild and by molecular classification as Class III (altered regulation), Class IV (altered conductance), or Class V (leading to exon skipping) [6], retain a critical level of CFTR function and generally develop a milder CF phenotype with chronic pancreatitis and pancreatic insufficiency developing significantly later than CF-severe patients [7]. However, because pancreatic function is retained, CF-mild patients are at risk for episodes of acute pancreatitis [8]. In addition to CF-severe and CF-mild phenotypes there are *CFTR* variants with CF-atypical phenotypes that do not cause classic CF, but are associated with some features of CF such as bronchiectasis, congenital absence of the vas deferens (CAVD), recurrent acute pancreatitis (RAP) or CP [9]. Little of the molecular pathophysiology of CF-atypical variants is known. Finally, there are many *CFTR* variants that are likely benign. Unfortunately, the overwhelming majority of the 1600 identified *CFTR* variants have not been completely studied or classified, especially with respect to potential effects on pancreatic duct cells and pancreatic phenotypes.

Serine peptidase inhibitor, Kazal type 1 (SPINK1), also known as pancreatic secretory trypsin inhibitor, is the first trypsin-regulating molecule in the pancreas recognized as an acute phase protein [10, 11]. Expression of SPINK1 is markedly up-regulated only after the initiation of inflammation [12]. As a specific and potent inhibitor of cationic and anionic trypsin, it prevents recurrent acute pancreatitis by preventing further activation of the acute inflammatory response through blocking trypsin [13]. Over 30 small case-control genetic linkage studies have been reported to evaluate the association between *SPINK1* N34S high-risk haplotype and chronic pancreatitis [14]. Despite evaluation of the same gene, the reported effect size ranged from odds ratios of non-significant to ~80 [14], suggesting that the biological effect of common genetic variants is complex.

Under normal conditions, trypsinogen is contained within two compartments while inside the pancreas: the acinar cell and the pancreatic duct. Since SPINK1 is synthesized and secreted in parallel to trypsinogen, functional genetic variants in *SPINK1* could affect trypsin regulation in either compartment. Regulation of trypsin in the acinar cell is largely dependent on regulation of intracellular calcium concentrations [15], while flushing of trypsin from the pancreatic duct is dependent on the function of the duct cell, in which CFTR is the central molecule for secretion of bicarbonate-rich juice.

ICP is strongly associated with the common *SPINK1* high-risk haplotype N34S [14], but since only a small minority of people who carry this variant develop CP we hypothesized that ICP patients with *SPINK1* variants must have a primary condition that increases the probability of recurrent intra-pancreatic trypsinogen activation. Defective CFTR-mediated fluid secretion from duct cells is such a condition, as occurs with CF-severe variants, but if there were *CFTR* defects that only affected bicarbonate conductance, then the effect may be limited to the pancreas - as predicted by our mathematical model [16] and recent molecular studies [17]. Thus, we hypothesize that defective pancreatic duct cell bicarbonate secretion would be associated with severe (type I or II) *CFTR* mutations (no bicarbonate or chloride conductance), or with *CFTR* mutations that selectively disrupt bicarbonate but not chloride conductance (A theoretical Class IVb, where “b” is a subtype of variant that selectively alters **b**icarbonate conductance). Either type of *CFTR* mutation would increase the risk of pancreatitis, but the latter would not be associated with a classic CF phenotype.

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To investigate the role of *CFTR* and *SPINK1* variants in idiopathic CP, we performed genotyping of the *CFTR* gene and *SPINK1* exon 3 in two independent populations of subjects with ICP (sporadic and familial) and two control populations. Finally, to identify a physiological reason for the repeated association of the CFTR variant p.R75Q with pancreatitis, we have tested the chloride and bicarbonate conductance of CFTR p.R75Q in a polarized epithelial cell line.

## Methods

### Study Population

The ascertainment of study patients was conducted as part of the multicenter North American Pancreatitis Study 2 (NAPS2) [18] and the University of Pittsburgh-based Molecular Genetics of Hereditary Pancreatitis (HP) study [19]. All study protocols were approved by the respective Institutional Review Boards. Pancreatitis phenotypes were determined by physicians specializing in pancreatic diseases using abdominal imaging findings of pancreatic fibrosis, calcifications, ductal dilation and atrophy. Hereditary pancreatitis, familial pancreatitis, and sporadic pancreatitis were defined according to Whitcomb [20]. Patients with alcoholic chronic pancreatitis were excluded, with alcoholic chronic pancreatitis defined by daily alcohol intake of 80 g per day in men and 25 g per day in women for more than 5 years, or evidence of chronic alcoholism using the TWEAK questions (old version) [21, 22]. Idiopathic recurrent acute pancreatitis was diagnosed by a minimum of two attacks of unexplained acute pancreatitis with amylase 3 times the upper limit of normal. Chronic pancreatitis was determined by imaging studies.

### *SPINK1* And *CFTR* Mutational Detection

Peripheral blood leukocyte DNA was purified as described [23, 24]. The *SPINK1* exon 3 with flanking intronic regions was sequenced to detect the pancreatitis-associated p.N34S and p.P55S polymorphisms as previously reported [24]. The entire *CFTR* coding region and the adjacent intronic regions were analyzed by DNA sequencing in all *SPINK1* mutation-positive subjects with pancreatitis. Amplification products were analyzed on an ABI 3700 DNA analyzer using POP-6 separation matrix and imported into Genotyper v3.7 software for allelic determination (Applied Biosystems). Mutation screening of the entire *CFTR* coding region was performed in the 95 healthy control subjects (control group 1) by Ambry Genetics (Costa Mesa, CA) using temporal temperature gradient gel electrophoresis (mTTGE). Exons 3, 9, 10 and IVS8 were sequenced in control group 2 (n=55) and in the 51 patients lacking a *SPINK1* mutation. To confirm sequencing data and expand the control population, all NAPS controls were tested for the specific *CFTR* mutations p.F508del and p.R75Q via custom iplex sequenom assay (primer sequences available on request).

### *CFTR* p.R75Q Patch Clamp Studies

The *CFTR* p.R75Q mutant was prepared using a Quickchange XL site-directed mutagenesis kit (Stratagene) from full-length human *CFTR* cDNA in pCDNA3.1. The *CFTR* p.R75Q mutation was introduced using polymerase chain reaction mutagenesis and verified via sequencing. (Primer sequences available on request) HEK 293 cells were transfected with *CFTR* WT or p.R75Q vectors using Lipofectamine 2000 (Invitrogen), and stable cell lines were selected in gentamycin (750 μg/ml). The cells were cultured in DMEM with 10% fetal bovine serum and were maintained in a humidified atmosphere containing 5% $CO_2$ at 37°C. The expression of *CFTR* WT and p.R75Q were confirmed by immunoblot, confirming similar expression levels. HEK 293 cells were harvested using TNTE buffer (50mM Tris HCl, 150mM NaCl, 1% Triton-100, 5 mM EDTA, supplemented with complete protease inhibitor cocktail, pH 7.5). Immunoblots were performed following a described protocol [25] using anti-CFTR mAb M3A7 (Chemicon).

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### Whole-cell Recording

Whole-cell voltage and current recordings were obtained from HEK 293 cells stably expressing CFTR WT or p.R75Q using an Axopatch 200B amplifier (Axon instruments, Foster City, California) and standard methods [26]. Cells were cultured on glass cover slips, and the recordings made 24–48 hours after plating. Pipettes were fire polished to a tip resistance of 2–5 MΩ in bath solution. Current-voltage curves were generated using voltage pulses of 200 ms duration over a range of −100 to +70 mV in 10 mV increments. CFTR gating was stimulated by addition of 10 μM forskolin and 100 μM cpt-cAMP to the bath perfusate (rate 3ml/min). Data acquisition and analysis were performed using pClamp software (version 9.0, Axon Instruments). All experiments were performed at 37°C. The recording solutions were: Pipette (mM): 90 K-glucomic acid, 35 KCl, 4.8 $NaH_2PO_4$, 1.03 $MgCl_2$, 5 Glucose, 0.5 EGTA; pH 7.2, titrated with KOH. The Bath(mM): 145 NaCl, 0.4 $KH_2PO_4$, 1.6 $K_2HPO_4$, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 5 Glucose; pH 7.2, titrated with NaOH. The $HCO_3^-$ containing Pipette (mM): 100 L-aspartic acid, 40 $NaHCO_3$, 100 CsOH, 2 NaCl, 10 TES, 1 EGTA; and the $HCO_3^-$ Bath (mM), 125 $NaHCO_3$, 18 NaCl, 2 $MgSO_4$, 30 Mannitol, 1 $CaCl_2.2H_2O$, 5 Glucose, 10 TES. Solutions containing 40 mM $HCO_3^-$ in the pipette were pre-gassed with 5% $CO_2$/95% air prior to filling the pipette, and bath solutions containing 125 mM $HCO_3^-$ were continuously gassed with 30% $CO_2$/70% air to maintain pH and $HCO_3$ concentration. 1 mM Mg-ATP and 100 μM Na-GTP were added to all pipette solutions. 5 μM CFTR $inh_{172}$ or 1 mM DIDS were added to the bath to test the blocker sensitivity of Cl and $HCO_3^-$ currents. All data are presented as mean±SEM. Results were analyzed and plotted using Clampfit 9.0 (Axon Instruments), SigmaPlot and Excel software.

### Statistical Analysis

All comparisons between observed counts of genotypes in cases and controls were done using a 2-sided Fisher's exact test (testing that the proportions observed in the cases were different from that observed in the controls). Comparison of age at onset data was done using a 2-sided non-parametric Wilcoxon rank-sum test to compare the distribution of ages at onset in the *SPINK1* mutation positive patient group with the *SPINK1* mutation negative patient group. No covariate corrections were made. Genotyping success rate was 100% in all sequenced samples, 99.4% and 98.6% for taqman genotyping of p.F508del and p.R75Q. All identified variants were tested for HWE fit and not found to deviate. For the frequency-based comparisons, the expected proportions of the different genotypes were derived using frequency data from our control populations. Comparisons between the expected proportions and the observed proportions were done using 2-sided binomial tests of proportions. Odds ratios were always generated from 2-sided Fisher's exact tests.

## Results

### Patient Population Data

A total of 197 patients and 653 control subjects were evaluated from the NAPS2 and familial pancreatitis cohorts (Figure 1). Of the 197 patients, we excluded 108 subjects who were alcoholics or had *PRSS1* mutations. From the familial pancreatitis cohorts, we excluded 9 related patients in our calculations, selecting one patient from each family who had been diagnosed first chronologically, resulting in a final study population of 80 ICP patients. The patients were then categorized by the inheritance pattern of disease, those with no affected relatives were called sporadic chronic pancreatitis (SP, n=53) and those with at least one affected family member were called familial pancreatitis (FP, n=27). The average age of onset of the SP and FP groups were 29 and 20 years, respectively. 38% of the SP group and 22% of the FP group were male. For control groups, 95 healthy adults over the age of 50 years (control group one) were investigated by CFTR screening of all 27 exons, and 55 spouses or unrelated friends of the pancreatitis patients (control group two) for exons

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3, 9, 10 and IVS8 (total control group=150), of which 36% were male. The remaining 503 control subjects were screened for *SPINK1* exon 3 variants and *CFTR* exon 3 and 10 variants.

### *SPINK1* Genetic Variants

Sequencing exon 3 of the *SPINK1* gene identified pancreatitis-associated p.N34S and p.P55S polymorphisms that have been described previously [24, 27]. *SPINK1* IVS3+2 T/C was identified in one patient and one control, so the intron variant was not included in the analysis. Twenty nine of eighty ICP patients were found to carry at least one *SPINK1* exon 3 variant (36%) (Table 1AI). Thirty-four percent of subjects with sporadic pancreatitis had *SPINK1* variants (p.N34S and/or p.P55S), as did 41% of probands from hereditary pancreatitis families. The onset of pancreatitis occurred at a median age of 13 years (±11.0 years, range 1–46 years) among *SPINK1*-positive subjects, compared with a median age of 34 years (±21.1 years, range 1–82 years) among those who were *SPINK1*-negative ($p<0.001$, Wilcoxon rank-sum test, 2-sided). *SPINK1*-negative SP patients had the highest median age of onset at 38 years.

*SPINK1* p.N34S and p.P55S variants were identified in 2 of 95 control subjects in group 1 (Table 1AII) and 3 of 55 in control group 2 (SFC). Comparison of these frequencies to those of all pancreatitis subjects (36%) demonstrates an overall odds ratio of 16.5 for the effect of *SPINK1* variants [p=<0.0001 by Fisher's exact test, 2-sided, Table 3 *SPINK1 only*, *Mut/any*]. Most of the effect was due to the more common *SPINK1* pN34S-associated haplotype. In addition, seven pancreatitis patients were found to carry 2 mutations in *SPINK1* as either p.N34S homozygotes or p.N34S/p.P55S compound heterozygotes, comparing this rate to that expected from the control population gives an overall odds ratio of 87.1 (95% CI 32.8–231, p=0.0005) for carriers of two *SPINK1* mutations.

### *CFTR* Mutation Identification And Characterization

To assess whether *CFTR* and *SPINK1* mutations are co-inherited in pancreatitis, we fully sequenced the *CFTR* gene of the patients that had previously identified mutations in *SPINK1*. Complete *CFTR* sequencing of the 29 *SPINK1* positive patients and the 95 samples in control group 1 not only revealed highly common SNPs but also eighteen rare sequence changes. Because of the high number of CFTR variants in this study and reported in the literature, we categorized the mutations into disease categories (Table 2). Two mutations, p.F508del and p.R560T, well characterized as Class II CFTR mutations, often associated with pancreatic insufficient CF, were categorized as CF-severe for statistical calculation. Also identified were six mutations (IVS8 T5, p.D443Y, p.G576A, p.F508C, p.I807M, p.M952T) reported to cause a milder form of CF or other CF related diseases (such as CAVD), which we have categorized as CF mild. While the IVS8 T5 variant has been categorized as class V, little is known about the functional consequences of the remaining mutations. Two peculiar mutations that occurred in both populations, c.1584GtoA (1716GtoA legacy name) and p.R75Q, have been generally regarded as benign sequence variations [28] (www.genetics.sickkids.on.ca) but repeatedly show association to CF-related diseases, pancreatitis [29–31] and some atypical cystic fibrosis patients [32]. Two individual non-synonymous sequence changes, p.R668C and p.I148T were identified with *CFTR* full sequencing in one control each, but without additional mutations found *in cis* (p.D443Y +p.G576A and c.3067del6 (i.e.3199del6), respectively). These isolated sequence changes are considered to be benign, and were categorized as "other". We categorized the remaining six sequence changes as "other" due to lack of any disease association or physiological data in either the literature or the *CFTR* mutation database (www.genet.sickkids.on.ca).

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### *CFTR* Genetic Variants Among Pancreatitis Patients

By direct sequencing of all 27 exons of *CFTR*, we identified variants in 69% of patients with *SPINK1* mutations (Table 1AI) (61% SP, 72% FP). Among the nine patients without *CFTR* mutations, four were homozygous or compound heterozygous for *SPINK1* mutations, which is often considered to be CP causing [27]. Mutation screening of all 27 exons in control group 1 (Table 1AII) revealed that 23% had *CFTR* sequence variants. In the *SPINK1* mutation positive patient group, CF-severe variants of *CFTR* (i.e. Class I or II) were more common than in controls (CF severe 17% vs 2%, p=0.002), as were CF-mild mutations (17% vs 3% p=0.007). All CF mutation groups were significantly more common in the patient population than control, except for those in the other category, which are either unassociated with disease or unknown (Other 7% vs 8% p=0.93).

To rapidly identify *CFTR* mutations in control group 2 and the *SPINK1* mutation negative patient group, we screened the *CFTR* exons that varied most commonly in the fully sequenced subjects. In patients with *SPINK1* mutations, *CFTR* variants were most commonly observed in exons 3 (e.g., p.R75Q), 10 (e.g., p.F508C, c.1584GtoA, p.F508del) and IVS8/exon9 (T5/TG12 or TG13) (Tables 2 and 3). Thus, we sequenced *CFTR* exons 3, 9, and 10 and IVS8 in subjects with idiopathic pancreatitis but without *SPINK1* mutations and a separate control group (55 spouses or friends of the pancreatitis subjects). *CFTR* variants in exons 3, 9, 10 and IVS8 were identified in 14.5% of spouse or friend controls and 27.5% of patients with idiopathic pancreatitis without *SPINK1* mutations. For the overall patient and control groups (irrespective of *SPINK1* mutation status) CF-severe mutations were identified significantly more often in patients as compared to controls (OR 7.1, p=0.009).

### Risk Analysis Of Individual *CFTR* Mutations

Recognizing that many SNPs in CFTR may be physiologically harmless and not cause disease, we calculated the individual risks of our most commonly identified CFTR variants p.F508del, p.R75Q, c.1584GtoA and IVS8 T5 for their effects on idiopathic chronic pancreatitis (see Table 3), regardless of *SPINK1* status. As expected, p.F508del was identified significantly more often in patients than controls (OR 2.75, p=0.02). The mild/atypical CF variant IVS8 T5 and the synonymous mutation c.1584GtoA were not significant (p=0.36 and p=0.12). The common CFTR variant p.R75Q, however, was significantly overrepresented in patients vs controls (OR=3.4, p=0.0002).

### Combined Effect Of *CFTR* And *SPINK1* Variants

We observed a striking increase in pancreatitis risk by comparing the expected with the observed frequency of combined *CFTR* and *SPINK1* mutations (OR 84.4, 95% CI 35.8–199, $p \ll 0.0001$, Table 3). The CF-severe *CFTR* mutation (p.F508del) when combined with a *SPINK1* variant, conferred the highest risk of pancreatitis (OR 131.5). Three control subjects were found to carry both a *SPINK1* variants and *CFTR* c.1584GtoA. When analyzed individually, the c. 1584GtoA mutation did not confer significant risk either with or without a corresponding *SPINK1* mutation, while the *CFTR* p.R75Q mutant conferred a significant risk for pancreatitis both when considered individually and with a concurrent *SPINK1* mutation (OR 3.4 and 62.5 – Table 3).

Given the carrier frequency of the aforementioned mutations, the expected frequency of a compound CFTR/SPINK1 genotype is approximately 5 in 1000. Of all 653 controls, one subject carried mutations in both *CFTR* and *SPINK1* concurrently (p.N34S/p.R75Q). A further review of the medical history of the control carrying both the *SPINK1* p.N34S and *CFTR* p.R75Q mutations revealed recurrent abdominal pain requiring hospitalization and an abdominal surgery, cholecystectomy. The subject was 55 years old at the time of

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ascertainment, an unrelated control subject, multiparous, a former smoker and self-reported a family and personal history of abdominal disease, raising the possibility that the individual had undiagnosed pancreatic disease.

### Anion Transport By p.R75Q CFTR In HEK 293 Cells

To assess the physiological properties of the p.R75Q variant, we stably expressed mutant and wild type CFTR in HEK293 cells and tested the relative conductances of each CFTR protein to $HCO_3^-$ and $Cl^-$. Figures 1A and 1B show the forskolin plus cpt-cAMP stimulated current-voltage (I–V) relations obtained in cells expressing either CFTR WT or p.R75Q and studied under conditions where chloride or $HCO_3$ are the major permeant anions. Currents in $Cl^-$ media at −60mV for CFTR WT and p.R75Q were not significantly different (mean −37.6 vs −28.6. p=0.3) (Figure 1C). When recorded using $HCO_3^-$ solutions in the pipette and bath, the current for CFTR WT was significantly less than in $Cl^-$ media, but significantly greater than that of the p.R75Q variant (mean −8.23 vs −1.53, p=0.0001). The $HCO_3$/Cl current ratio for CFTR WT was 0.22, in agreement with published values of $HCO_3$/Cl permeability. Viewed in another way (Figure 1C), the current ratio in $Cl^-$ media for p.R75Q/WT was 0.76, which was not statistically different from 1.0; however, the current ratio in bicarbonate media for p.R75Q/WT was 0.18, and the $HCO_3$/Cl current ratio for p.R75Q was 0.053, four-times lower than that for CFTR WT.

## Discussion

In the present study of ICP in patients without clinical evidence of CF, we confirmed that the combination of trans-heterozygous *CFTR* and *SPINK1* variants markedly increases the risk of pancreatitis, as first observed by Noone et al [33]. However, we demonstrate for the first time a very high risk of pancreatitis in patients with specific *CFTR* mutations (p.R75Q, p.F508del) and show that CFTR bicarbonate conductance is specifically impaired in the relatively common *CFTR* variant p.R75Q.

Much work has been done on the genetics of pancreatitis, and although the association of each the genes *PRSS1*, *SPINK1* and *CFTR* remains generally supported, the relationship between these genes still remains unresolved. Our study demonstrated a strong association between *SPINK1* and *CFTR* mutations in pancreatitis, which is in agreement with the data of some investigators [34, 35] while the data present by others showed no association [36–38]. Our patient population may different in some ways from that of conflicting reports since it encompasses a wide range of European ethnic backgrounds [18], and thereby diminishes the likelihood that unusual local genetic or environmental factors might affect the results. Furthermore, our study population includes probands of subjects with familial pancreatitis, further strengthening the likelihood of identifying genetic interactions. While our study clearly establishes a pathological association between bicarbonate-limiting *CFTR* mutations and *SPINK1* mutations, other risk factor may also be important in ICP.

The majority of our patients with idiopathic sporadic and idiopathic familial pancreatitis that were heterozygous for *SPINK1* variants were also heterozygous for *CFTR* variants. In these patients without *CFTR* variants, about half had homozygous or compound heterozygous *SPINK1* variant genotypes, a condition that may be sufficient to cause CP directly [27]. The high risk of polygenic *CFTR-SPINK1* variants is also suggested by a much earlier age of onset than seen in sporadic CP without mutations in these genes, suggesting that unidentified environmental factors, rather than strong genetic factors, are associated with disease expression in older patients (e.g. moderate alcohol consumption, smoking or other toxins).

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The *CFTR* p.R75Q variant was associated with a high risk of pancreatitis in the presence of a *SPINK1* mutation in the present study, and it was also observed in the series reported by Noone [33], Weiss [36] and Tzetis [34] in patients with CP. While this variant has been investigated previously for causing CF [28], a number of researchers have determined that *CFTR* p.R75Q is processed and matures much like CFTR WT in cells, and physiological studies show no gating or $Cl^-$ channel dysfunction [39]. This type of data strengthens the argument that .PR75Q is a sequence variant, not contributing to the autosomal recessive disorder CF [28] (www.genetics.sickkids.on.ca). However, the fact that *CFTR* p.R75Q is repeatedly reported as a *CFTR* variant in a number of atypical CF patients [32] and CF-related disorders such as sarcoidosis [29], chronic obstructive pulmonary disease (COPD) [30] and CP [31] suggests that normal function is disrupted in some way. Here we demonstrate for the first time that p.R75Q alters bicarbonate, but not chloride conductance. This is consistent with our CP model that recognizes CFTR as pancreatic duct cell bicarbonate channel, and predicts that *CFTR* mutations disrupting bicarbonate conductance will markedly increase risk of pancreatic disease either through total disruption of protein processing (e.g. p.F508del) or alteration in channel properties (e.g. p.R75Q) [16]. This finding also suggests that there may be additional *CFTR* mutations that specifically disrupt bicarbonate conductance and thus are also specific risk factors for CP but not CF. In 2001, Choi et al. reported bicarbonate conductance testing of 16 CFTR mutations, showing that chloride and bicarbonate conductance dysfunctions are not often linked to each other and are independently mutation specific [40]. Our investigation of bicarbonate specific mutations in *CFTR* is ongoing.

Our data suggest that the correlation of the relatively common *CFTR* variant p.R75Q with a *SPINK1* mutation increases the risk of chronic pancreatitis in a multiplicative manner (*SPINK* alone OR=16.5, p.R75Q alone OR=3.4, combined with SPINK1 OR=62.5, Table 3), while that of heterozygous *CFTR* p.R75Q or *CFTR* p.F508del variants becomes insignificant in a *SPINK1* WT background. This suggest an epistatic association between SPINK1 and CFTR.

Our findings also confirm reports that compound *SPINK1* variant genotypes confer very high risk for pancreatitis, as first suggested by Witt and colleagues [27] and confirmed by Pfützer and colleagues [24]. In addition, we found that the majority of subjects with heterozygous *SPINK1* mutations and pancreatitis also have *CFTR* mutations, providing compelling evidence of a polygenic, high-risk pathway leading to ICP [14, 24, 41]. This pancreas-specific mechanism associated with ICP was found to be common in both sporadic and familial populations. We further identified a new class of *CFTR* variants that appear to be pancreas-specicific because of their selective effect on bicarbonate secretion (suggested Class IVb). Finally, this study illustrates the importance of combining our knowledge of biology with multiple candidate gene testing and functional experimentation to help recognize and understand complex and multiplicative risks for common, complex disorders.

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## References

1. Etemad B, Whitcomb DC. Chronic pancreatitis: Diagnosis, classification, and new genetic developments. Gastroenterology. 2001; 120:682–707. [PubMed: 11179244]

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2. Bockman DE. Morphology of the exocrine pancreas related to pancreatitis. Microscopy Research & Technique. 1997; 37:509–19. [PubMed: 9220428]

3. Shrikhande SV, Martignoni ME, Shrikhande M, Kappeler A, Ramesh H, Zimmermann A, Buchler MW, Friess H. Comparison of histological features and inflammatory cell reaction in alcoholic, idiopathic and tropical chronic pancreatitis. Br J Surg. 2003; 90:1565–72. [PubMed: 14648737]

4. Durie PR. Inherited and congenital disorders of the exocrine pancreas. Gastroenterologist. 1996; 4:169–87. [PubMed: 8891682]

5. Augarten A, Tov AB, Madgar I, Barak A, Akons H, Laufer J, Efrati O, Aviram M, Bentur L, Blau H, Paret G, Wilschanski M, Kerem B-S, Yahav Y. The changing face of the exocrine pancreas in cystic fibrosis: the correlation between pancreatic status, pancreatitis and cystic fibrosis genotype. European Journal of Gastroenterology & Hepatology. 2008; 20:164–168.10.1097/MEG. 0b013e3282f36d04 [PubMed: 18301294]

6. Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet. 2003; 67:471–85. [PubMed: 12940920]

7. Stern RC, Eisenberg JD, Wagener JS, Ahrens R, Rock M, doPico G, Orenstein DM. A comparison of the efficacy and tolerance of pancrelipase and placebo in the treatment of steatorrhea in cystic fibrosis patients with clinical exocrine pancreatic insufficiency. Am J Gastroenterol. 2000; 95:1932–8. [PubMed: 10950038]

8. De Boeck K, Weren M, Proesmans M, Kerem E. Pancreatitis Among Patients With Cystic Fibrosis: Correlation With Pancreatic Status and Genotype. Pediatrics. 2005; 115:e463–469. [PubMed: 15772171]

9. Ockenga J, Stuhrmann M, Ballmann M, Teich N, Keim V, Dork T, Manns MP. Mutations of the cystic fibrosis gene, but not cationic trypsinogen gene, are associated with recurrent or chronic idiopathic pancreatitis. Am J Gastroenterol. 2000; 95:2061–7. [PubMed: 10950058]

10. Ogawa M. Pancreatic secretory trypsin inhibitor as an acute phase reactant. Clin Biochem. 1988; 21:19–25. [PubMed: 2449986]

11. Lasson A, Borgstrom A, Ohlsson K. Elevated pancreatic secretory trypsin inhibitor levels during severe inflammatory disease, renal insufficiency, and after various surgical procedures. Scand J Gastroenterol. 1986; 21:1275–80. [PubMed: 3810002]

12. Khalid A, Finkelstein S, Thompson B, Kelly L, Hanck C, Godfrey TE, Whitcomb DC. A 93 year old man with the PRSS1 R122H mutation, low SPINK1 expression, and no pancreatitis: insights into phenotypic non-penetrance. Gut. 2006; 55:728–31. [PubMed: 16354799]

13. Aoun E, Muddana V, Papachristou GI, Whitcomb DC. SPINK1 N34S is strongly associated with recurrent acute pancreatitis but is not a risk factor for the first or sentinel acute pancreatitis event. Am J Gastroenterol. 2010; 105:446–51. [PubMed: 19888199]

14. Aoun E, Chang CC, Greer JB, Papachristou GI, Barmada MM, Whitcomb DC. Pathways to injury in chronic pancreatitis: decoding the role of the high-risk SPINK1 N34S haplotype using meta-analysis. PLoS ONE. 2008; 3:e2003. [PubMed: 18414673]

15. Sutton R, Criddle D, Raraty MG, Tepikin A, Neoptolemos JP, Petersen OH. Signal transduction, calcium and acute pancreatitis. Pancreatology. 2003; 3:497–505. [PubMed: 14673201]

16. Whitcomb DC, Ermentrout GB. A mathematical model of the pancreatic duct cell generating high bicarbonate concentrations in pancreatic juice. Pancreas. 2004; 29:E30–E40. [PubMed: 15257112]

17. Park HW, Nam JH, Kim JY, Namkung W, Yoon JS, Lee JS, Kim KS, Venglovecz V, Gray MA, Kim KH, Lee MG. Dynamic regulation of CFTR bicarbonate permeability by [Cl−]i and its role in pancreatic bicarbonate secretion. Gastroenterology. 2010; 139:620–31. [PubMed: 20398666]

18. Whitcomb DC, Yadav D, Slivka A, et al. Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). Pancreatology. 2008; 8:520–531. [PubMed: 18765957]

19. Applebaum-Shapiro SE, Finch R, Pfützer RH, Hepp LA, Gates L, Amann S, Martin SP, Ulrich CD II, Whitcomb DC. Hereditary Pancreatitis in North America: The Pittsburgh - Midwest Multi-Center Pancreatic Study Group Study. Pancreatology. 2001; 1:439–443. [PubMed: 12120221]

20. Whitcomb, DC. Hereditary Diseases of the Pancreas. In: Yamada, T.; Albers, DH.; Laine, L.; Kaplowitz, N.; Owyang, C.; Powell, DW., editors. Textbook of Gastroenterology. Philadelphia: Lippincott, Williams and Wilkins; 2003. p. 2147-2165.

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21. Chan AW, Pristach EA, Welte JW, Russell M. Use of the TWEAK test in screening for alcoholism/heavy drinking in three populations. Alcohol Clin Exp Res. 1993; 17:1188–92. [PubMed: 8116829]
22. Schneider A, Barmada MM, Slivka A, Martin JA, Whitcomb DC. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta(1), interleukin-10, and interferon-gamma polymorphisms in patients with alcoholic chronic pancreatitis. Alcohol. 2004; 32:19–24. [PubMed: 15066699]
23. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nature Genetics. 1996; 14:141–5. [PubMed: 8841182]
24. Pfutzer RH, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, Furey WF, Whitcomb DC. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. Gastroenterology. 2000; 119:615–23. [PubMed: 10982753]
25. Sun F, Hug MJ, Bradbury NA, Frizzell RA. Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. J Biol Chem. 2000; 275:14360–6. [PubMed: 10799517]
26. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch. 1981; 391:85–100. [PubMed: 6270629]
27. Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, Landt O, Becker M. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nature Genetics. 2000; 25:213–6. [PubMed: 10835640]
28. Zielenski J, Bozon D, Kerem B, Markiewicz D, Durie P, Rommens JM, Tsui LC. Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics. 1991; 10:229–35. [PubMed: 1710599]
29. Schurmann M, Albrecht M, Schwinger E, Stuhrmann M. CFTR gene mutations in sarcoidosis. Eur J Hum Genet. 2002; 10:729–32. [PubMed: 12404105]
30. Divac A, Nikolic A, Mitic-Milikic M, Nagorni-Obradovic L, Petrovic-Stanojevic N, Dopudja-Pantic V, Nadaskic R, Savic A, Radojkovic D. High frequency of the R75Q CFTR variation in patients with chronic obstructive pulmonary disease. Journal of Cystic Fibrosis. 2004; 3:189–191. [PubMed: 15463907]
31. Cohn JA, Neoptolemos JP, Feng J, Yan J, Jiang Z, Greenhalf W, McFaul C, Mountford R, Sommer SS. Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers. Human Mutation. 2005; 26:303–307. [PubMed: 16134171]
32. Hughes D, Dork T, Stuhrmann M, Graham C. Mutation and haplotype analysis of the CFTR gene in atypically mild cystic fibrosis patients from Northern Ireland. Journal of Medical Genetics. 2001; 38:136–139. [PubMed: 11288718]
33. Noone PG, Zhou Z, Silverman LM, Jowell PS, Knowles MR, Cohn JA. Cystic fibrosis gene mutations and pancreatitis risk: relation to epithelial ion transport and trypsin inhibitor gene mutations. Gastroenterology. 2001; 121:1310–9. [PubMed: 11729110]
34. Tzetis M, Kaliakatsos M, Fotoulaki M, Papatheodorou A, Doudounakis S, Tsezou A, Makrythanasis P, Kanavakis E, Nousia-Arvanitakis S. Contribution of the CFTR gene, the pancreatic secretory trypsin inhibitor gene (SPINK1) and the cationic trypsinogen gene (PRSS1) to the etiology of recurrent pancreatitis. Clin Genet. 2007; 71:451–7. [PubMed: 17489851]
35. Keiles S, Kammesheidt A. Identification of CFTR, PRSS1, and SPINK1 mutations in 381 patients with pancreatitis. Pancreas. 2006; 33:221–7. [PubMed: 17003641]
36. Weiss FU, Simon P, Bogdanova N, Mayerle J, Dworniczak B, Horst J, Lerch MM. Complete cystic fibrosis transmembrane conductance regulator gene sequencing in patients with idiopathic chronic pancreatitis and controls. Gut. 2005; 54:1456–60. [PubMed: 15987793]
37. Garg PK, Khajuria R, Kabra M, Shastri SS. Association of SPINK1 Gene Mutation and CFTR Gene Polymorphisms in Patients With Pancreas Divisum Presenting With Idiopathic Pancreatitis. Journal of Clinical Gastroenterology. 2009; 9:848–852. [PubMed: 19593166]

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38. Sobczynska-Tomaszewska A, Bak D, Oralewska B, Oracz G, Norek A, Czerska K, Mazurczak T, Teisseyre M, Socha J, Zagulski M, Bal J. Analysis of CFTR, SPINK1, PRSS1 and AAT mutations in children with acute or chronic pancreatitis. J Pediatr Gastroenterol Nutr. 2006; 43:299–306. [PubMed: 16954950]

39. Gené GG, Llobet A, Larriba S, Semir Dd, Martínez I, Escalada A, Solsona C, Casals T, Aran JM. N-terminal CFTR missense variants severely affect the behavior of the CFTR chloride channel. Human Mutation. 2008; 29:738–749. [PubMed: 18306312]

40. Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO3− transport in mutations associated with cystic fibrosis. Nature. 2001; 410:94–7. [PubMed: 11242048]

41. Threadgold J, Greenhalf W, Ellis I, Howes N, Lerch MM, Simon P, Jansen J, Charnley R, Laugier R, Frulloni L, Olah A, Delhaye M, Ihse I, Schaffalitzky de Muckadell OB, Andren-Sandberg A, Imrie CW, Martinek J, Gress TM, Mountford R, Whitcomb D, Neoptolemos JP. The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. Gut. 2002; 50:675–81. [PubMed: 11950815]

42. Welsh, JW.; Ramsey, BW.; Accurso, F.; Cutting, GR. Cystic fibrosis. In: Scriver, CR.; Beaudet, AL.; Valle, D.; Sly, WS., et al., editors. The metabolic and molecular bases of inherited disease. 8. New York: McGraw Hill; 2001. p. 5121-88.

43. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. Annual Review of Genetics. 1995; 29:777–807.

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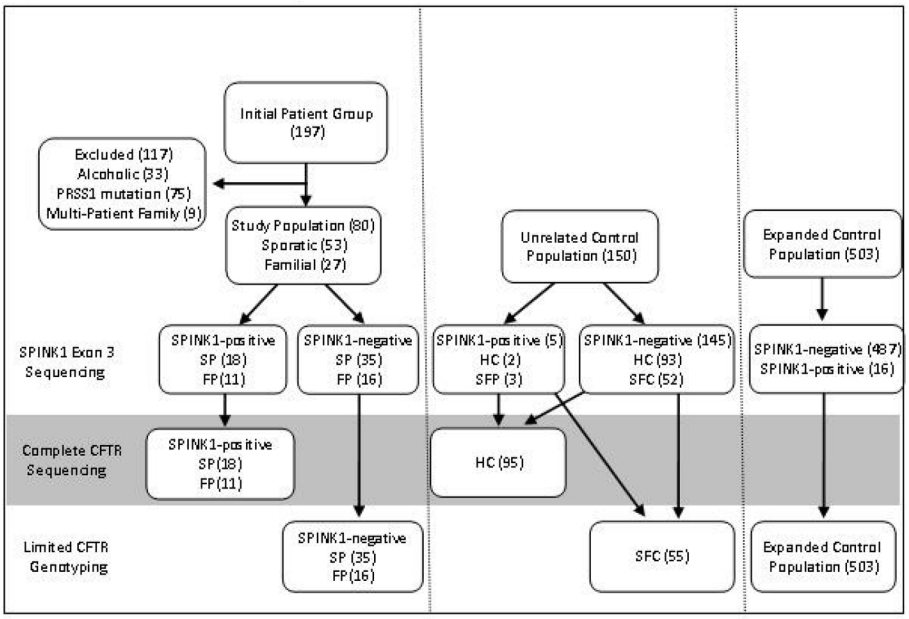


**Figure 1. Ascertainment of Patient and Control Populations**

Classification of subjects studied according to phenotype and genotype. PRSS1, protease serine 1; SP Sporadic Pancreatitis; FP, familial pancreatitis family proband; HC, healthy controls; SFC, spouse or friend control; NAPS controls, final control group limited to SNP specific genotyping.

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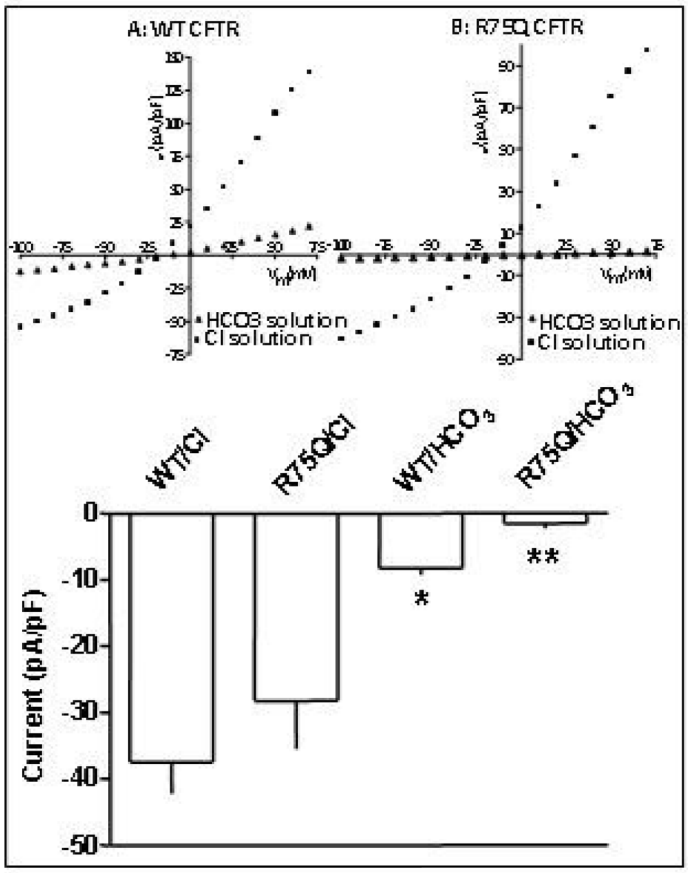


**Figure 2. The R75Q mutation results in a selective decrease in CFTR $HCO_3$ conductance**

(A) Current-voltage (I–V) relations recorded under whole-cell patch clamp conditions from HEK 293 cells stably expressing WT CFTR with pipette and bath solutions containing predominantly Cl or $HCO_3$ in both pipette and bath solutions, as indicated in Methods. The mean data are from 8 and 9 recordings from cells exposed to the Cl and $HCO_3$ recording conditions, resp. (B) Whole-cell I–V relations from HEK cells stably expressing R75Q CFTR. Recording conditions and numbers are the same as in A. (C) Mean whole-cell currents recorded using Cl and $HCO_3$ solutions at a pulse voltage of −60 mV from the data summarized in A and B. (*) indicates a significant mean current difference in $HCO_3$ vs. Cl media; (**) additional significant difference between currents in $HCO_3$ media comparing WT and R75Q CFTR expressing cells.

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**Table 1**

**CFTR mutation class types and corresponding disease severity**

CFTR mutations identified in our study and their corresponding exons are arranged by most current understanding of disease association. CF mutation class has been defined in only 3 of 18 variants.

| CFTR Mutation | Exon | CF Mutation Class | Disease Association | % carriers case (N) | % carriers controls (N) |
|---|---|---|---|---|---|
| **p.R75Q** | **3** | | **“CP”** | **16.2 (80)** | **5.8 (653)** |
| **c.1584GtoA (p.E528E)** | **10** | | **“CP”** | **8.7 (80)** | **3.3 (150)** |
| **p.F508del** | **10** | II | **CF severe** | **8.7 (80)** | **3.4 (653)** |
| p.R560T | 11 | II | CF severe | 3.4 (29) | 0 (95) |
| **IVS8-T5/TG12or13** | **i8** | V | **CF mild** | **5.0 (80)** | **3 (150)** |
| **p.F508C** | **10** | | **CF mild** | **1.2 (80)** | **0 (150)** |
| p.I807M | 13 | | CF mild | 3.4 (29) | 0 (95) |
| **p.D443Y**+G576A+R668C* | **9**;12;13 | | CF mild | 3.4 (29) | 0 (95) |
| p.G576A+R668C* | 12;13 | | CF mild | 0 (29) | 1 (95) |
| p.M952T | 15 | | CF mild | 3.4 (29) | 0 (95) |
| p.R668C | 13 | | other | 0 (29) | 1 (95) |
| c.3139+42AtoT | i17a | | other | 3.4 (29) | 0 (95) |
| p.N1432K | 24 | | other | 0 (29) | 1 (95) |
| c.-9CtoT | 1 | | other | 0 (29) | 1 (95) |
| **p.C76W** | **3** | | other | 0 (80) | 0.7 (150) |
| p.I148T | 4 | | other | 0 (29) | 1 (95) |
| c.2657+22GtoA | i14b | | other | 0 (29) | 1 (95) |
| p.T1086A | 17b | | other | 0 (29) | 1 (95) |

** p.R75Q and c.1584GtoA have been reported to be associated with CP, although not categorically.

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## Table 2A

### Total CFTR sequencing results of ICP patients with SPINK1 mutations and Healthy Controls

**I.** Patients (1–29) with their corresponding categories (Dx) Sporadic (SP) or Familial (FP) Pancreatitis, ages of diagnosis (age dx) and CFTR, SPINK1 genotyping results.
**II.** Healthy controls (1–95), subjects 22–95 had no identified mutation in either CFTR or SPINK1.

| I. Patients With SPINK1 Mutations | | | | |
|---|---|---|---|---|
| | **Dx** | **Age Dx** | **CFTR mutations** | **SPINK1 mutations** |
| 1 | SP | 12 | −/− | N34S/P55S |
| 2 | SP | 46 | −/− | N34S/P55S |
| 3 | SP | 13 | −/− | N34S/N34S |
| 4 | FP | Infant | −/− | N34S/N34S |
| 5 | FP | 8 | R560T/− | N34S/P55S |
| 6 | FP | 15 | M952T/− | N34S/N34S |
| 7 | SP | 19 | +R75Q/− | N34S/N34S |
| 8 | SP | 3 | +F508del/− | P55S/− |
| 9 | SP | 3 | +F508del/1584GtoA | N34S/− |
| 10 | SP | 19 | +F508del/− | N34S/− |
| 11 | FP | 12 | +F508del/I807M, 3139+42AtoT | N34S/− |
| 12 | SP | 14 | D443Y+G576A+R668C* | N34S/− |
| 13 | SP | 1 | +F508C/− | N34S/− |
| 14 | SP | 20 | +IVS8-T5-TG12/− | N34S/− |
| 15 | SP | 16 | +R75Q/− | P55S/− |
| 16 | SP | 9 | +R75Q/− | N34S/− |
| 17 | SP | 9 | +R75Q/− | N34S/− |
| 18 | SP | 16 | +R75Q/+1584GtoA | N34S/− |
| 19 | FP | 7 | +R75Q/− | N34S/− |
| 20 | FP | 35 | +R75Q/− | N34S/− |
| 21 | FP | 2 | +1584GtoA/− | N34S/− |
| 22 | FP | Child | +1584GtoA/− | N34S/− |
| 23 | SP | 14 | +1584GtoA/− | N34S/− |
| 24 | FP | 14 | 3139+42AtoT/− | N34S/− |
| 25 | FP | 28 | −/− | N34S/− |
| 26 | FP | 36 | −/− | N34S/− |
| 27 | SP | 8 | −/− | N34S/− |
| 28 | SP | 9 | −/− | N34S/− |
| 29 | SP | 3 | −/− | N34S/− |

| II. Healthy Controls | | |
|---|---|---|
| | **CFTR mutations** | **SPINK1 mutations** |
| 1 | −/− | N34S/− |

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| **II. Healthy Controls** | | |
|---|---|---|
| | **CFTR mutations** | **SPINK1 mutations** |
| 2 | 1584GtoA/− | N34S/− |
| 3 | F508del/− | −/− |
| 4 | F508del/− | −/− |
| 5 | G576A+R668C/− | −/− |
| 6 | IVS8 T5-TG12/− | −/− |
| 7 | R75Q/− | −/− |
| 8 | R75Q/− | −/− |
| 9 | R75Q/− | −/− |
| 10 | R75Q/− | −/− |
| 11 | R75Q/− | −/− |
| 12 | R75Q/− | −/− |
| 13 | R75Q/− | −/− |
| 14 | R75Q/− | −/− |
| 15 | R75Q/− | −/− |
| 16 | −9CtoT/− | −/− |
| 17 | +C76W/− | −/− |
| 18 | T1086A/− | −/− |
| 19 | R668C/− | −/− |
| 20 | N1432K/− | −/− |
| 21 | I148T/− | −/− |
| 22 | 2657+22GtoA | −/− |
| **23–95** | **−/−** | **−/−** |

* mutations commonly found *in cis*

+ Denotes mutations in exons that were chosen for CFTR screening of the larger population

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**Table 2B**

**Total Targeted CFTR and SPINK1 Genotyping Results**

Carrier rates of each mutation in SPINK1 (N34S, P55S, any) or CFTR (F508del, R75Q, 1584GtoA, T5-TG12) are represented as a fraction of total subjects genotyped and (columns 2, 3). Carrier rates of CFTR mutations within the subset of SPINK1 mutation carriers are expressed in fraction of carriers (columns 4, 5) and fraction of total subjects (columns 6,7)

| Mutation | Patients (%) | Controls (%) | Patients w/SPINK1 mut(%) | Controls w/SPINK1 mut(%) | Co-inheritance Patients (%) | Co-inheritance Controls (%) |
|---|---|---|---|---|---|---|
| N34S | 27/80 (33.8) | 20/653 (3.1) | | | | |
| P55S | 5/80 (6.2) | 1/653 (0.2) | | | | |
| any SPINK1 | 29/80 (36.2) | 21/653 (3.2) | 7/29 (24.1) | 0/21 | | |
| F508del | 7/80 (8.8) | 22/653 (3.4) | 4/29 (13.8) | 0/21 | 4/80 (5.0) | 0/653 (0) |
| R75Q | 13/80 (16.2) | 35/653 (5.7) | 6/29 (20.7) | 1/21 (4.7) | 7/80 (8.8) | 1/653 (0.2) |
| 1584GtoA | 7/80 (8.8) | 28/653 (4.3) | 5/29 (17.2) | 3/5 (60.0) | 4/80 (5.0) | 3/150 (2.0) |
| IVS8 T5-TG12 | 4/80 (5.0) | 4/150 (2.7) | 1/29 (3.4) | 0/5 | 1/80 (1.2) | 0/150 (0) |

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**Table 3**

Statistical Analysis of *SPINK1* and *CFTR* Genotyping Data

| CFTR[1] | SPINK1[2] | Patient Carriers | Patient non- carriers | Control carriers[3] | Control Non carriers[3] | OR | 95% CI | p-value |
|---|---|---|---|---|---|---|---|---|
| SPINK1 only | | | | | | | | |
| | Mut/any | 29 | 51 | 5 | 145 | **16.5** | 6.1–44.9 | **<<0.0001** |
| | Mut/Mut | 7 | 73 | 11* | 9989* | **87.1** | 32.8–231 | **0.0005** |
| CFTR only – full sequencing results | | | | | | | | |
| All variants | | 20 | 9 | 22 | 73 | **7.4** | 2.3–18.5 | **<<<0.0001** |
| CF Severe | | 5 | 24 | 2 | 93 | **9.7** | 1.8–53.0 | **0.002** |
| CF Mild | | 5 | 24 | 3 | 92 | **6.4** | 1.4–28.6 | **0.007** |
| Other | | 2 | 27 | 7 | 88 | 0.9 | 0.2–4.8 | 0.93 |
| CFTR analysis of individual mutations | | | | | | | | |
| F508del/any | | 7 | 73 | 22 | 631 | **2.75** | 1.1–6.6 | **0.02** |
| R75Q/any | | 13 | 67 | 35 | 618 | **3.43** | 1.7–6.8 | **0.0002** |
| 1584GtoA/any | | 7 | 73 | 5 | 145 | 2.8 | 0.9–9.0 | 0.12 |
| IVS8 T5-TG12/any | | 4 | 76 | 4 | 146 | 1.9 | 0.5–7.9 | 0.45 |
| Combined effects of SPINK1 and CFTR mutations | | | | | | | | |
| F508del/any | Mut/any | 4 | 76 | 4* | 9996* | **131.5** | 32.2–535.6 | **0.013** |
| R75Q/any | Mut/any | 7 | 73 | 1 | 653 | **62.5** | 16.6–95.4 | **<<<0.0001** |
| 1584GtoA/any | Mut/any | 5 | 75 | 3 | 147 | 3.27 | .76–14.0 | 0.09 |
| IVS8 T5-TG12/any | Mut/any | 1 | 79 | 9* | 9991* | 14.0 | 1.7–112.2 | 0.348 |

[1] CFTR genotypes categorized according to severity of CFTR phenotype [42, 43]

[2] SPINK1 N34S and/or P55S heterozygous/homozygous/compound carriers all considered (Mut/any). Homozygous or compound heterozygotes only (Mut/Mut).

[3] Control populations counts for the compound CFTR and joint CFTR-SPINK1 model denoted with (*) were calculated by multiplying corresponding rates from the individual (SPINK1/CFTR only) populations and multiplying by 10,000 – e.g. expected proportions of F508del-SPINK1$^{(+)}$ carriers is given by (2/150)*(5/150) = 0.0004, or 4/10,000.